

Macrophage-Based Combination Therapies as a New Strategy for Cancer Immunotherapy

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Keywords

Macrophages · Phagocytosis checkpoints · Tumor-associated macrophages · Macrophage-based cell therapies

Abstract

Background: Cells of the immune system can inhibit tumor growth and progression; however, immune cells can also promote tumor cell growth, survival, and angiogenesis as a result of the immunosuppressive microenvironments. In the last decade, a growing number of new therapeutic strategies focused on reversing the immunosuppressive status of tumor microenvironments (TMEs), to reprogram the TME to be normal, and to further activate the antitumor functions of immune cells. Most of the “hot tumors” are encompassed with M2 macrophages promoting tumor growth, and the accumulation of M2 macrophages into tumor islets leads to poor prognosis in a wide variety of tumors. **Summary:** Therefore, how to uncover more immunosuppressive signals and to reverse the M2 tumor-associated macrophages (TAMs) to M1-type macrophages is essential for reversing the immunosuppressive state. Except for reeducation of TAMs in the cancer immunotherapy, macrophages as central effectors

and regulators of the innate immune system have the capacity of phagocytosis and immune modulation in macrophage-based cell therapies. **Key Messages:** We review the current macrophage-based cell therapies that use genetic engineering to augment macrophage functionalities with antitumor activity for the application of novel genetically engineered immune cell therapeutics. A combination of TAM reeducation and macrophage-based cell strategy may bring us closer to achieving the original goals of curing cancer. In this review, we describe the characteristics, immune status, and tumor immunotherapy strategies of macrophages to provide clues and evidences for future macrophage-based immune cell therapies.

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Introduction

Macrophages, as the member of the mononuclear phagocytic system, can perform distinct functional phenotypes depending on tumor microenvironment (TME) cues. It is generally known that macrophages were de-

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fined into 2 polarized phenotypes, the classically activated M1 macrophages versus the alternatively activated M2 macrophages [1]. As macrophages reside in the TME, various signals activate extensively intracellular transcriptional cross talk in macrophages and lead to polarization of functions ranging from proinflammatory responses to inflammatory decomposition [2–4].

Macrophages in tumor do not develop directly from residential macrophages in tissues. They are recruited from monocytes in peripheral blood circulation [5]. A growing number of studies have suggested that the characteristics of TAMs are not the same as M2 macrophages [6, 7]. The culture supernatant of the primary tumor can cause the phenotype and function of macrophages to shift to mixed M1/M2 polarized phenotypes from M1 or M2 macrophages completely [8]. In vitro, TAMs also can express interleukin-6 (a proinflammatory cytokine), whereas the expression of interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) is lower. Compared with M2 macrophages, the expression of CD163 and TGF- β in TAMs is reduced by a 10th, which shows that TAMs may be a group of mixed macrophages inclined to M2 macrophages [9]. The phenotype of TAMs is significantly different in variant types of tumors and even in different parts of the same tumor. Therefore, according to the heterogeneity of the tumor cells, customized treatment strategies should be adopted for different cancers and patients.

In recent years, immune checkpoint inhibitors (ICIs) have shown good clinical efficacy in tumor immunotherapy. In 2011, the anticytotoxic T lymphocyte-associated protein 4 (CTLA-4) antibody ipilimumab for metastatic melanoma was approved by the US Food and Drug Administration as the first listed ICI [10]. In 2014, another ICI nivolumab was approved as the world's first publicly available programmed death-1 (PD-1) inhibitor [11–13]. In the following years, multiple ICIs were approved for several cancer species, including metastatic non-small cell lung cancer, gastric cancer, and liver cancer, which have substantially improved patient prognoses across multiple metastatic and treatment-refractory cancers [14–16]. The phagocytosis of macrophages is critical for bridging the innate and adaptive immunity activation in immunotherapy [17, 18]. Normal tissues and cells have the intrinsic ability to avoid self-elimination by phagocytes through the expression of antiphagocytosis molecules. However, cancer cells cunningly exploit the antiphagocytosis mechanisms to evade immune-mediated eradication. The identification and therapeutic targeting of phagocytosis checkpoints in cancer might provide a promising avenue for the development of cancer immu-

notherapies to eliminate tumor immune escape. Thus, targeting phagocytosis checkpoints of macrophages in the TME has been an issue of close attention for reversing the immunosuppressive status of TAMs (shown in Fig. 1).

In addition to exploit immune checkpoint mechanisms, another way to reverse the immunosuppressive status is genetically editing macrophages ex vivo and transplanting them for macrophage-based cell therapies [19–25]. Engineered macrophages in vitro injected into patients mobilize the activation of the TME, stimulating the activity of T cells in vivo and modulating the immunosuppressive state ultimately [26, 27]. During the early stages of the adoptive macrophage cell therapy, only a small number of macrophages were recruited to the tumor sites and might transform from an antitumor phenotype into the immunosuppressive status in the TME [26, 28–30]. Novel methods need to be developed to make adoptively transferred macrophages resistant to the TME and stay constitutively in an antitumor status.

Development and Function of Macrophages in Tumor Progression

Macrophages as innate immunocytes inhabit different tissues, with considerable heterogeneity. Despite the diverse residences, based on the polarization, macrophages have a series of general functions, including tissue remodeling, regulation of inflammation, induction of immunity, thrombosis, and various forms of endocytosis [31, 32]. Macrophage polarization is constantly in flux at any point in an inflammatory process. IFN- γ produced by Th1 cells in combination with TNF- α or the activation of Toll-like receptors (TLRs) by bacterial cell wall components such as lipopolysaccharides (LPSs) can induce macrophages to become “classically activated” M1 macrophages. M1 macrophages in the TMEs can secrete proinflammatory mediators such as TNF- α , IL-1 β , IL-12, IL-23, reactive oxygen, and nitrogen species and transform the immunosuppressive state of the TME via stimulating the cytotoxicity of T cells [33, 34]. In contrast, macrophage polarization by activated Th2 cell-derived IL4 or IL13 produces an alternative set of cytokines and chemokines that oppose the repertoire of classically activated M1 macrophages, and these “alternatively activated” macrophages are designated as M2 macrophages [1, 35]. M2 macrophages generally express higher levels of scavenger receptors and proangiogenic factors [36, 37]. TAMs display an M2-like phenotype, including anti-inflammation, vascularization, and downregulated immunity [38].

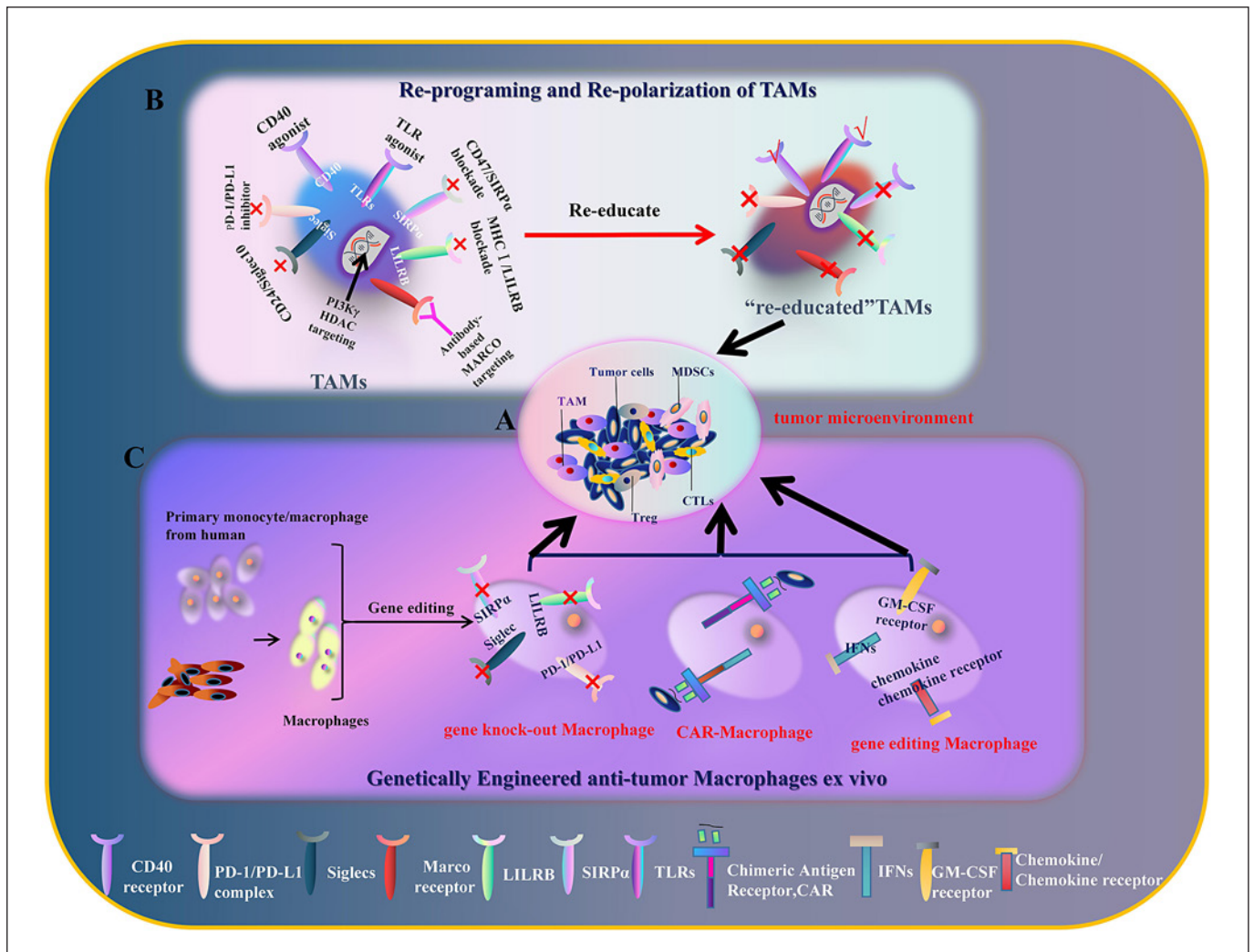


Fig. 1. Macrophage-based combination immunotherapies in cancer. **A** Macrophages are prominent in different tumor microenvironments of all types of malignancy. In solid tumors, macrophages can represent up to 50% of the mass, becoming the main immune population. **B** Reprogramming and repolarization of tumor-associated macrophages can improve the phagocytosis in certain types of cancer. **C** In addition to immunotherapeutic strategies to focus on reeducating immunosuppressive TAMs or enhancing macro-

phage phagocytosis, another potential immunotherapeutic approach associated with macrophages is the application of genetic engineering to augment macrophage behaviors or endow new antitumor therapeutic functionalities. TAMs, tumor-associated macrophages; CAR, chimeric antigen receptors; PD, programmed death; CSF, colony-stimulating factor; SIRP, signal regulatory protein; TLR, Toll-like receptor; HDAC, histone deacetylase.

Macrophages are prominent in different TMEs of all types of malignancy. In solid tumors, macrophages can represent up to 50% of the mass as the main immunocyte population [39]. Monocytes are recruited to the tumor tissues through blood vessels throughout tumor progression, promoting vascularization of primary tumor nodules, invasiveness, and the degree of malignancy of advanced tumors. Multiform cytokines and chemokines such as colony-stimulating factor-1 (CSF-1), the CC che-

mokines, CCL2, CCL3, CCL4, CCL5, and CCL8, stromal cell-derived factor 1 (SDF-1/CXCL12), and vascular endothelial growth factor (VEGF) derived from tumors and cancer-associated fibroblasts can recruit macrophages to infiltrate tumors [40–42].

Generally, functions of TAMs in the TME can be described as follows: (1) the growth factors secreted by TAMs promote the proliferation and invasion of cancer cells, such as the epidermal growth factor (EGF) pro-

duced by TAMs, and promote the invasion of cancer cells, and VEGF secreted by TAMs regulates cancer cell invasion and angiogenesis. Besides, CSF-1 generated by tumors can recruit macrophages infiltration so that the accumulation of EGF, VEGF, MMP-9, and TGF- β produced by TAMs can further augment the invasion of cancer cells [43–45]. (2) TAMs can promote neoangiogenesis through secreting proangiogenic chemokines and proteolytic enzymes, such as MMPs and cathepsins, or expressing Tie-2 which can bind angiopoietins (Ang-1, Ang-2, etc.). Reconstitution of tumor-bearing Mmp9-knockout (KO) mice by transplantation of wild-type, MMP-9-competent hematopoietic cells caused the reappearance of MMP-9-positive TAMs and restoration of tumor angiogenesis and metastasis [46, 47]. Tie^{high} TAMs can significantly promote angiogenesis of tumors, and upon depletion of Tie^{high} TAMs, angiogenesis and tumor growth can be inhibited such as glioma, pancreatic cancer, and breast cancer in mice [48, 49]. (3) TAMs aggravate the immunosuppression of TME through the secretion of immunosuppressive mediators (TGF- β , IL-10, PGE2, etc.) to hamper the antitumor effect of the host immune system. Blocking IL-10, as well as other immunosuppressive cytokines present in the TME, may complement therapeutic strategies for antitumor immune responses [50]. Immunosuppressive molecules prevent the expression of MHC II on macrophages from antigen presentation to T cells. For instance, IL-10 can stimulate the expression of the E3 ubiquitin ligase March-I in activated macrophages, thereby downregulating MHC-II, CD86, and antigen presentation to T cells [51].

Strategies to Target TAMs to Improve Antitumor Immune Responses

In recent years, cancer immunotherapy has been focused on strengthening or improving the immune activation mechanism – developing various types of immunotherapy based on known immune molecular mechanisms, and promoting immune activation by controlling immune regulation to improve the effectiveness of antitumor immune responses. Exploiting immune cells with antitumor activity, such as adoptive T cells or natural killer (NK) cells, to replace immune cells that no longer have powerful functions *in vivo*, or to stimulate the activation of immune cells *in vivo* by tumor vaccine, have achieved the purpose of eliminating tumors [52].

However, cancer cells not only rely on “cancerous growth” to resist immune defense, but they also actively

adopt various strategies to impede antitumor immunity – these strategies are collectively referred to as “immune evasion mechanisms.” These tactics always destroy the intrinsic antitumor immunity and lead to loss of control of tumor growth. The immune evasion mechanisms continue to develop during cancer progression and become complicated in advanced cancer. Since the Food and Drug Administration approval of the CTLA-4 antibody ipilimumab for metastatic melanoma in 2011, ICIs have substantially improved patient prognoses across multiple metastatic and treatment-refractory cancers [10, 53–55]. So far, PD-1/L1 antibody therapy has shown efficacy outcomes of >15 cancer treatments, and the survival rates of melanoma and advanced non-small cell lung cancer have increased more than threefolds. However, the effective rate of PD-1 antibody in most patients with advanced tumors is only 20%, and the drug resistance appears in the course of treatment. Therefore, it is crucial to focus on innate immune cell therapy and develop more effective phagocyte targets while searching for new adaptive immune checkpoints to improve the response rate of tumor immunotherapy [56–58].

Phagocytosis Checkpoints of Macrophages as New Targets for Cancer Immunotherapy

Cells of the myeloid lineage are the most abundant immune cells in the body originally, monocytes enter tumors through blood vessels throughout the life span of tumors, from early-stage tumor nodules that are beginning to vascularize to late-stage tumors that are invasive and metastatic, and macrophages, in particular, have a remarkable potential as mediators of anticancer therapies based on their robust ability to perform phagocytosis [59]. Macrophages gradually change from the initial M1 phagocytic state to M2 immunosuppressive state promoting the proliferation of tumor cells [60–62]. Macrophages possess multiple immunosuppressive receptors that initiate inhibitory phagocytosis signaling after binding to ligands at the surface of tumor cells [61, 63]. The successful application of CD47 or PD1 antibody prompts us that targeting inhibitory signals of different tumors and exploring more immunosuppressive receptors in macrophages are essential to reverse the immunosuppressive state of the solid TME [59, 64–66].

At present, there are >30 sorts of inhibitory receptors in macrophage cells, which can be divided into 2 categories according to their structural characteristics. A class of immunoglobulin superfamily (IgSF) genes belonged to

type I transmembrane proteins are mostly located in 19q13, and most of them contain immunoreceptor tyrosine-based inhibitory motif (ITIM), an immune receptor with (I/L/V) XYXX (L/V) sequence [67]. These receptors mainly include FcγRII, Ig-like transcripts, signal regulatory protein (SIRP), paired immunoglobulin-like receptor, killer receptors, CD22, and PD-1. The other class is the C-type lectins superfamily-coding gene, which is located at 12p13 and belongs to type II transmembrane protein. It mainly includes the Ly49 family, NKG2 family, CD94, CD72, and dendritic cell (DC) immunoreceptor. These receptors are expressed in the form of disulfide-bonded homodimers or heterodimers with extracellular recognition of carbohydrate-recognition domain whose ligand recognition processes are calcium-dependent. Since FcγRIIb was reported as the first ITIM, ITIM-dependent inhibitory receptors and their respective ligands have been demonstrated to play a role in the control of various cell activities including NK cell responses (killer receptor-MHC class I ligands), phagocytosis mediated by macrophages (SIRPα-CD47), and adaptive T- and B-cell responses (CTLA-4-CD80/CD86 and PD-1-PD-L1/2) [67].

The CD47-SIRPα Axis

Identified in the late 1990s, SIRPα was found to be expressed on myeloid cells, with 3 extracellular Ig-like regions [68–70]. The cytoplasmic region contains 4 ITIM regions, which would be phosphorylated through extracellular domains connecting to ligands. The phosphorylated ITIM mediates the aggregation and activation of tyrosine phosphatase SHP1 and SHP2. Activated SHP1 and SHP2 in turn dephosphorylate some specific protein substrate to attenuate signaling induced by ITAM and eventually form negative regulation (shown in Fig. 2). CD47 is a ligand of SIRPα, which is an extensive transmembrane glycoprotein with an N-terminal IgV-like region, 5 transmembrane regions, and a selectable intracellular binding region. A more recent study has confirmed the therapeutic efficacy of anti-CD47 monoclonal antibodies (mAbs) in eliminating small cell lung cancer xenograft in mice when used in combination with anti-CD56 mAbs [71]. In various xenograft tumor models, human CD47-blocking mAbs were proven to have excellent efficacy against solid and hematological malignancies [72–74].

In 2014, the first agent targeting the CD47-SIRPα axis proceeded to clinical trials. So far, >10 anti-CD47 agents entered the clinical treatment, and clinical outcomes have shown the excellent data of partial agents, such as Hu5F9-G4, TTI-621, CC90002, and ALX148 [75–78]. However,

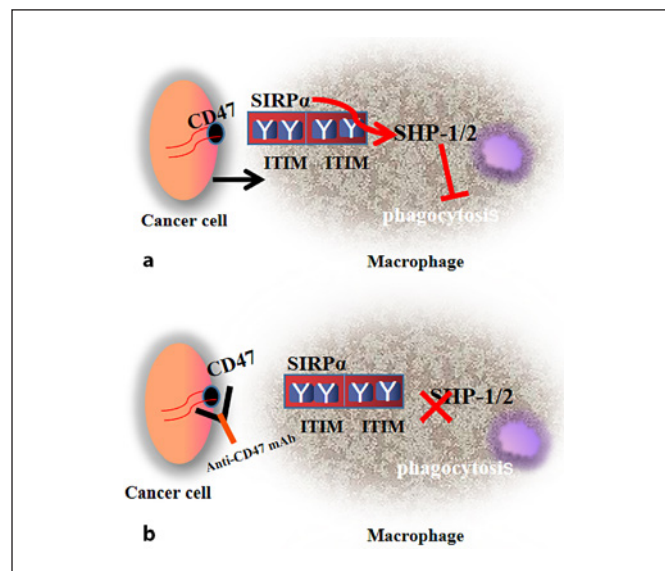


Fig. 2. The CD47-SIRPα axis. CD47 is highly expressed on multiple types of tumor cells. The phagocytosis is downregulated by the regulation of the inhibitory signal by the SHP-1 through which CD47 ligands bind to SIRPα, which is an inhibitory receptor expressed on macrophages. **a** When CD47 binds to SIRPα, the phosphorylated ITIM mediates the aggregation and activation of tyrosine phosphatase SHP1/SHP2, which inhibits phagocytosis via downstream mediators. **b** Blockade of the CD47/SIRPα axis using phagocytosis checkpoint strategies, such as antibodies, recombinant proteins, or engineered macrophages, attenuates the inhibitory signal to promote the phagocytosis of macrophages. SIRP, signal regulatory protein; ITIM, immunoreceptor tyrosine-based inhibitory motif; mAb, monoclonal antibody.

CD47 treatments may accidentally injure red blood cells, which also express CD47 and lead to anemia [79–81]. Due to the increase of “eat me” signals such as calmodulin (calreticulin) on the surface of aging red blood cells, the “eat me” signal exceeds the “don’t eat me” signal of CD47, which promotes the phagocytosis of macrophages to the aging red blood cells. The classical CD47 mAb includes active domain F(ab’)₂ that binds specifically to SIRPα and Fc segments that cause cytotoxic effects. The intense cytotoxicity of the Fc segment is bound to attack a large number of normal red blood cells, whereas the CD47-SIRPα signaling pathway to exert biological effects without Fc segment is not sufficient to kill tumor cells [82, 83]. In addition to modifying antibody molecules, adjusting the dosage and manner of treatment is important to reduce the incidence of anemia. The researchers developed a combination of “trigger dose” and “therapeutic dose” treatment regimens that incipient administration of short-term low-dose Hu5F9-G4 in combination with

Table 1. Clinical trials investigating phagocytosis checkpoint blockade

Drug	Target	Conditions	Strategy	Start date	Phase	NCT number
AO-176	CD47	Lymphoma AML Solid tumor	Single agent	February 2019	Phase 1	NCT03834948
IBI187	CD47	Advanced malignancies	Single agent	January 2019	Phase 1	NCT03763149
SRF231	CD47	AML Solid tumor	Single agent or combination with rituximab	April 2018	Phase 1	NCT03512340
ALX148	CD47	Metastatic cancer Solid tumor Non-Hodgkin lymphoma	Single agent combination with pembrolizumab or trastuzumab or tuximab	February 2017	Phase 1	NCT03013218
Hu5F9-G4	CD47	Myelodysplastic syndromes AML	Single agent or combined with azacitidine	September 2017	Phase 1	NCT03248479
Hu5F9-G4	CD47	Solid tumors	Single agent combined with cetuximab	November 2016	Phase 1/2	NCT02953782
TTI-621	CD47	Hematological malignancies solid tumor	Single agent combined with rituximab or nivolumab	January 2016	Phase 1	NCT02663518
CC-90002	CD47	Leukemia, myeloid, and acute myelodysplastic syndromes	Single agent	March 2016	Phase 1	NCT02641002
CC-90002	CD47	Advanced solid and hematological Malignancies	Single agent or combined with rituximab	March 2015	Phase 1	NCT02367196
NC318	Siglec 15	Solid tumors	Single agent	December 2018	Phase 1/2	NCT03665285
JTX-8064	LILRB2	Solid tumors	Single agent or combination with an anti-PD-1/PD-L1 agent	Pre-IND	–	–
Anti-LILRB4 antibody	LILRB4	AML	Single agent	Pre-IND	–	–

PD, programmed death; AML, acute myeloid leukemia.

rituximab, which triggered predictable and transient mild anemia, followed by the generation of new red blood cells, with the macroeffect of changing the overall age of red blood cells from older to younger, reducing the incidence of anemia [80].

The MHC-I-LILRB Axis

The promising clinical outcomes of the blockade of the CD47-SIRPα interaction confirm that targeting the immunosuppressive checkpoint of the CD47-SIRPα axis promotes the phagocytosis of macrophages [72, 73, 84]. Nevertheless, many cancer patients are irresponsive to the immunotherapeutic strategy for targeting CD47-SIRPα due to the heterogeneity of tumors; therefore, more phagocytosis checkpoint inhibitors are developed for patients as much as possible [59] (shown in Table 1).

The LILRB is a group of type I transmembrane glycoproteins with extracellular Ig-like domains that bind ligands MHC-I, which is a class of inhibitory receptors expressed on myeloid cells (shown in Fig. 3). The receptors

consisted of ITIM generate an immunosuppressive signal after tyrosine phosphorylation as a result of MHC-I of tumor cells and LILRB in macrophages interaction, leading to the blockade of macrophage phagocytosis. The expression of the common MHC class I component β2-microglobulin (β2M) by cancer cells directly protected them from phagocytosis. Thus, the treatment of blocking MHC class I molecules or LILRB1 can enhance the phagocytosis of macrophages [85, 86]. In patients with tumors that have normal or high expression of MHC class I, agents directed at the MHC class I-LILRB1 axis might facilitate an antitumor immune response and could potentially act together with agents directed against CD47 or SIRPα.

Treatment with a blocking mAb to LILRB2 can reeducate TAM to improve the therapeutic effect of cancer immune therapies through the modulation of the TME. Researchers developed novel LILRB2-specific mAbs and found that a subset of LILRB2 antagonists altered AKT-dependent maturation of macrophages in response to macrophage CSF and enhanced NF-κB and STAT1 acti-

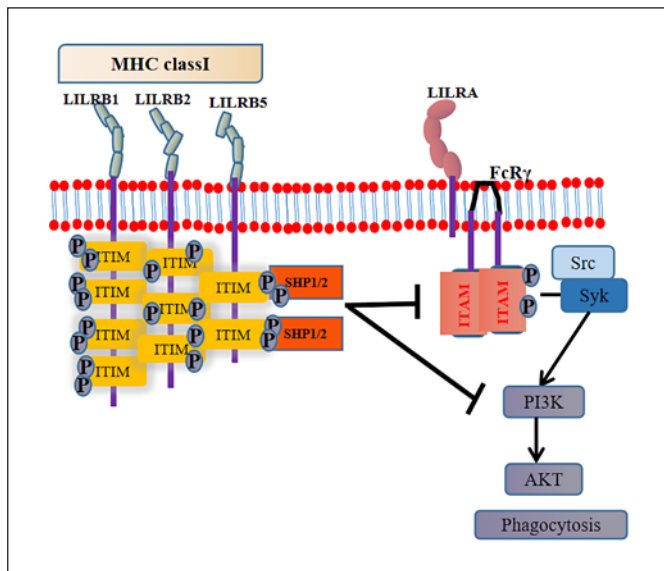


Fig. 3. The MHC-I-LILRB axis. The LILR family comprises a set of paired immunomodulatory receptors expressed among human myeloid and lymphocyte cell populations. The LILRB is a group of type I transmembrane glycoproteins with extracellular Ig-like domains that bind ligands MHC-I, which is a class of inhibitory receptors expressed on myeloid cells. The receptors consisted of ITIM generate an immunosuppressive signal after tyrosine phosphorylation as a result of MHC-I of tumor cells and LILRB in macrophage interaction, leading to the blockade of macrophage phagocytosis. LILR, leukocyte immunoglobulin-like receptor; ITIM, immunoreceptor tyrosine-based inhibitory motif.

vation in response to LPS/IFN- γ stimuli [87]. Multiple immunosuppressive checkpoints exist in immune cells and have been found that the combined application of immune checkpoints can enhance the phagocytosis of macrophages [88, 89].

Siglecs Axis

Siglec is a lectin surface receptor molecule that binds sialic acid chain, consisting of a V-type amino-terminal immunoglobulin domain to regulate sialic acid recognition as well as several different immunoglobulin domains. According to its sequence similarity and evolutionary conservatism, Siglec is divided into 2 categories: the first includes Sn (sialic acid adhesin, siglec-1), CD22 (siglec-2), MAG (siglec-4), and siglec-15, and the second type is CD33-related Siglec, in which Siglec-5, -6, -7, -8, -9, -10, -11, -14, and -16 exist in the human body, and Siglec-E, -F, -G, and -H exist in rodent animals [90–92]. Siglecs are similar to other immunosuppressant receptors in various cells of the immune system, except for Siglec-4, which contain one or more ITIMs in the cytoplasmic domain

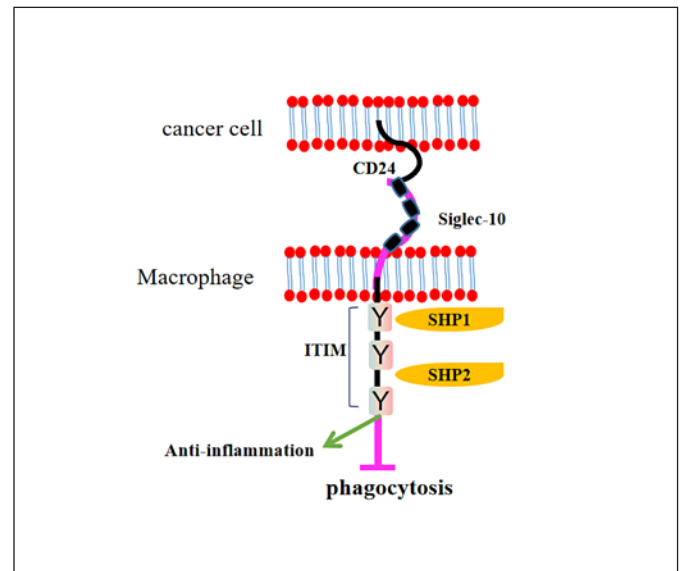


Fig. 4. The CD24/Siglec-10 axis. Siglec10 is similar to other immunosuppressant receptors in various cells of the immune system, which contains ITIMs in the cytoplasmic domain. Siglec-10 binds its ligand CD24 on cancer cells, and the intracellular ITIMs domains recruit the tyrosine phosphatases SHP-1 and SHP-2 and thus attenuates the phagocytosis of macrophages. Genetic ablation of CD24 or Siglec-10 by monoclonal antibodies, as well as blocking CD24-Siglec-10 interactions, can significantly enhance phagocytosis in all human tumors expressing CD24. ITIM, immunoreceptor tyrosine-based inhibitory motif.

[93]. After the surface sialylated sugar chain of the tumor cell binds to the macrophages, the intracellular ITIM domains recruit the tyrosine phosphatases SHP-1 and SHP-2 and thus regulate the cells of the innate and adaptive immune responses [94].

Earlier studies on Siglec focused on the expression of CD33 in monocytes, neutrophils, and myeloid progenitor cells. Gemtuzumab ozogamicin, an mAb specifically targeting CD33, was approved for clinical treatment of acute myeloid leukemia in 2002 [95]. At present, there are still many CD33-related tumor immunotherapies in the clinic. Recent studies have shown that other members of the Siglec family play a significant role in autoimmune diseases, inflammatory responses, and tumors by regulating innate and adaptive immune responses. Therefore, targeting Siglec family may become a new direction of tumor immunotherapy. Many tumors overexpress CD24, while TAMs expressed high levels of Siglec-10 [96]. Genetic ablation of CD24 or Siglec-10 by mAbs, as well as blocking CD24-Siglec-10 interactions, can significantly enhance phagocytosis in all human tumors expressing CD24 (shown in Fig. 4).

Siglec-15 is a member of the sialic acid-binding immunoglobulin-like lectin family (Siglec family) as a macrophage-associated T-cell suppressive molecule. It was suggested that Siglec-15 is closely related to the B7 gene family and may have the same immunomodulatory function as the member of the B7 family [97]. Gene ablation or antibody blockade of Siglec-15 can convert an immune-suppressive TME to an inflammatory site in some tumor models, which suggests that Siglec-15 is a potential candidate for normalization cancer immunotherapy. The expressions of Siglec-15 and PD-L1 are mutually exclusive, targeting Siglec-15 may be optimum treatment on tumors for the patients that are irresponsive to anti-PD-1/PD-L1 therapy. Currently, a first-in-human phase I clinical trial is ongoing to test the effect of a humanized mAb (NC318) on Siglec-15 in solid tumors (NCT03665285).

Antibody-Dependent Cellular Phagocytosis

The administration of targeted antibodies to improve the phagocytosis has been an effective therapeutic strategy to enhance the antitumor immune responses of macrophages. mAbs bind a variety of FcγRs by the Fc segment and perform their effector functions, including antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and complement-dependent cytotoxicity [98, 99]. For instance, the Fc region of IgG1 activates the cytotoxicity of NK cells to induce ADCC by binding to FcγRIII (CD16A), triggers ADCP through binding to FcγRIII (CD16A), FcγRII (CD32A), and FcγRI (CD64) on macrophages, and activates the complement cascade via binding to C1q, resulting in complement-dependent cytotoxicity. Enhancing the phagocytosis of TAMs as effector cells targeting tumor cells through mAb therapy promotes TAMs to exhibit antitumor activities in TME [100–103]. The anti-CD20 mAb rituximab was one of the first drugs that were approved for clinical use to treat B-cell malignancies [104, 105]. Various antitumor mAbs have been applied for cancer through enhancing ADCC and ADCP, such as the anti-EGFR mAbs cetuximab and panitumumab to treat head and neck cancer and metastasized colorectal carcinoma and the anti-HER-2 mAb trastuzumab to treat breast carcinoma [106, 107]. TAM infiltration in the invasive breast carcinomas generates poor prognosis of patients, whereas patients with colorectal cancer had a better prognosis when colon carcinomas were densely infiltrated with macrophages [108, 109]. Thus, malignancy with TAMs is not always accompanied by a poor prognosis

[6, 110]. The study found that TAMs can accelerate the proliferation of tumor cells indeed from mouse breast carcinomas in *in vitro* 3D assays; nonetheless, these TAMs had the ability of phagocytosis with Fcγ receptors in the presence of anti-CD142 mAbs [111]. The anti-CD142 human IgG1 mAb can bind strongly to CD142 (tissue factor) expressed on the breast tumor cells and then mediate the phagocytosis of TAMs entirely through Fc-dependent killing. Also, the deficiency of TAMs in mice reduced the efficiency of anti-CD142 mAb therapy to prevent breast carcinoma outgrowth and metastases.

The mononuclear phagocytic system, as the main effector cells, can rapidly clear tumor cells in the peripheral blood and slowly clear tumor cells in lymph nodes, splenic nodules, abdominal cavity, and Payer's collective lymph nodes [112]. Depletion of B cells by anti-murine CD20 mAbs was strictly dependent on the mononuclear phagocyte network and required expression of activating Fcγ receptors. Moreover, when mice were injected with lymphoma cells after depletion of macrophages by clodronate liposomes, the therapeutic effect of mAbs was significantly reduced, while deficiency of NK cells and neutrophils in mice rarely impacts the efficacy of mAbs [113]. Activated monocytes/macrophages can produce cytokines (IL-1, IL-6, IL-12, TNF-α, etc.), chemokines (MIP, MCP, IL-8, etc.), proteolytic enzymes, and reactive oxygen species to enhance the phagocytosis function. Mutation of anti-CD20 mAbs that did not interact with C1q also has the antitumor effect, demonstrating that survival of lymphoma-bearing mice after mAb therapy was not dependent on complement activation, but on tumor cell elimination by macrophages via Fcγ receptor-mediated processes [112]. In addition, the occurrence of several hematological malignancies, including Hodgkin disease and anaplastic large cell lymphoma, is accompanied by high expression of CD30. The anti-CD30 mAb SGN-30 induces direct antitumor activity through antibody-dependent cellular phagocytosis, mediated by macrophages, to inhibit the growth of CD30⁺ tumor cells. Depletion of macrophages reduced survival of tumor-bearing mice treated with SGN-30; in contrast, ablation of NK cells did not significantly affect efficacy [102].

The antitumor performance of mAb is dependent on the recruitment and phagocytosis function of TAMs. The therapeutic effect of anti-Her2 Ab was restricted by TAMs with the M2-type function. Tumor local delivery of IL-21 can skew TAM polarization from the M2 to the M1 phenotype, which reverses immunosuppression and invokes the active antitumor potential to bridge the gap between the innate immunity and T-cell responses for tumor re-

gression. The combination of anti-Her2/neu mAb and IL-21 efficiently reverses the polarization of TAMs for promoting the therapeutic effect of anti-Her2 Ab [114]. However, there is evidence suggesting that the ADCP activity of M2 macrophages is more notable than M1 macrophages in the presence of antilymphoma mAb. M-CSF and IL-10-differentiated M2-type macrophages increased significantly the phagocytic capacity of macrophages toward rituximab-opsonized target cells [100]. M-CSF or IL-10 increases the phagocytic capacity of macrophages toward apoptotic cells or pathogens and may be related to the higher activity of M2 macrophages in scavenging debris and tissue remodeling [115, 116]. It suggests that IL-10-derived M2c (IL-10) cells are much more phagocytic than IL-4-differentiated M2a (IL-4) cells also for Ab-opsonized target cells [117]. Thus, manipulation of TAM functional polarization may be a promising strategy for the mAb treatment.

Targeting the Cellular Metabolism for TAM Reprogramming

Metabolic reprogramming is an indispensable process of macrophage plasticity and polarization. Once macrophages are recruited to the TME, they are obliged to undergo metabolic adaptations to survive in the harsh tumor milieu. For instance, arginine metabolism is associated with macrophage function, which is a feature of macrophage polarization. M1 macrophages use arginine as a substrate for iNOS, converting arginine into NO and citrulline by iNOS. The produced NO subsequently suppresses oxidative phosphorylation (OXPHOS) through the inhibition of enzymes involved in the TCA and electron transport chain and upregulates glycolysis. Instead, M2 macrophages utilize arginine as a substrate for Arg1. The metabolism of M1 macrophages is characterized by increased glycolytic flux and reduced mitochondrial OXPHOS. In contrast, oxidative glucose metabolism (fatty acid oxidation) is the metabolic pathway favored by IL-4-activated M2 macrophages. Investigating the metabolic changes between the TME and the TAMs is a key process of exploring novel therapeutic approaches, targeting the metabolic reprogramming of macrophages to enhance the phagocytosis of the innate immune cells.

Glucose Metabolism of the TAMs

The stimulation of TLR4 by LPS induces macrophage polarization to the M1 type, switching its major glucose metabolism pathway from OXPHOS to glycolysis. Be-

sides, macrophages can also be induced to express hypoxia-inducible factor 1 α (HIF-1 α), initiating the glycolysis process to produce ATP. HIF-1 α can promote the expression of proinflammatory genes in macrophages and enhance phagocytosis. Deletion of HIF-1 α attenuates the ability of phagocytosis and proinflammatory cytokine secretion of macrophages [118]. HIF-1 α contributes to the synthesis of iNOS and the enhancement of other hypoxia element-dependent enzyme transcriptional activity under LPS stimulation or hypoxia [119]. Chelidonic acid in M1 macrophages can bind to propyl hydroxylase to inhibit the dehydrogenation and degradation of HIF-1 α , and exogenous succinic acid can promote the stable expression of HIF-1 α and enhance the glycolytic metabolism of macrophages [120].

Hypoxic TAMs acquire angiogenic and immunosuppressive properties. Hypoxia is one of the major factors that determine the vascular structure of solid tumors. Hypoxia blocks the mechanistic target of rapamycin (mTOR) function through HIF-mediated transcriptional induction of the mTOR complex 1 (mTORC1) inhibitor REDD1 (regulated in development and DNA damage response 1), inhibiting glycolysis under hypoxic conditions. On the other hand, REDD1-deficient TAMs compete with adjacent endothelial cells for glucose by activating mTOR to promote the process of glycolysis, thus endowing the normalization of vascular structure and inhibiting tumor metastasis [121].

Lipid Metabolism of the TAMs

Besides glucose metabolism, the studies of lipidomics confirm that lipid metabolism is associated with functional reprogramming of TAMs [122]. Lipid metabolism can administer to the macrophage phagocytosis by regulating membrane fluidity and provide energy for the process. However, the excess absorption of cholesterol leads to abnormal cholesterol metabolism in macrophages, which can induce a series of pathological changes. Plenitudinous endoplasmic reticulum membrane and free cholesterol promote the esterification reaction of cholesterol acyltransferase 1 (ACAT1), which in turn leads to more free cholesterol production and increases inflammatory signals through lipid rafts, especially TLR and NF- κ B signals [123–125]. The levels of fatty acid absorption and fatty acid oxidation increased significantly in IL-4-stimulated M2 macrophages and were inhibited in M1 macrophages. Peroxisome proliferator-activated receptor (PPAR) is a ligand-dependent transcript that acts as a fatty acid receptor to regulate glucose and lipid metabolism. It can be divided into PPAR α , PPAR δ , and PPAR γ

subpopulations in which PPAR α /PPAR γ is widely expressed in human and mouse monocytes, inhibiting the expression of proinflammatory genes in macrophages. PPAR γ promotes fatty acid β -oxidation and mitochondrial production at the gene transcription level to induce the polarization of M2 macrophage. PGC-1 β is a PPAR transcriptional costimulatory factor that increases the expression of genes associated with fatty acid oxidation and promotes OXPHOS in cells. Conditional knockdown of PGC-1 β genes inhibits intracellular OXPHOS levels as well as M2 macrophage functions and greatly facilitates the proinflammatory responses of LPS-activated M1 macrophages [126].

Two recent studies also showed lipid accumulation in TAMs led to macrophage activation toward an M2-like phenotype. Deficiency of monoacylglycerol lipase (MGLL) results in lipid overload in TAMs. MGLL knock-out in macrophages attenuated IL-1 β and TNF- α mRNA expression in response to IFN- γ and LPS stimulation and potentiated the expression of IL-10, Arg-1, and TGF- β in response to IL-4 treatment in BMDMs. MGLL inhibits CB2 cannabinoid receptor-dependent tumor proliferation, and also MGLL deficiency promotes CB2/TLR4-dependent macrophage activation, which further suppresses the function of tumor-associated CD8 $^{+}$ T cells. Xiang et al. [127] utilize MGLL as a switch for CB2/TLR4-dependent macrophage activation to provide potential targets for cancer therapies. They screened the triglyceride metabolism-associated enzymes in TAMs and found that the expression of abhydrolase domain containing 5 (ABHD5), an activator of triglyceride hydrolysis, is lower in migratory TAMs than in the nonmigratory TAMs. ABHD5 deficiency in macrophages results in lipid accumulation and promoted NF- κ B p65-dependent production of matrix metalloproteinases. Thus, the outcomes showed that macrophage ABHD5 inhibits matrix metalloproteinase-dependent cancer cell migration [127, 128].

Combination of Antibodies and Agonists to Reprogram TAMs

The single treatment of targeting to the phagocytosis checkpoints can block inhibitory receptor signaling on TAMs and promote phagocytosis effectively, but not completely nor universally. The patients who are irresponsive with single immunotherapy agents or resistant to the treatments are still inappropriate with the application of ICIs in the clinical treatment. The combined application of phagocytosis checkpoint inhibitors and repo-

larization agents can promote TAMs as M1 macrophages with antitumor function, which is more effective than the single strategy for reeducating macrophages.

CD40/CD40L

CD40 is a highly conserved costimulatory receptor, mainly expressed on antigen-presenting cells, such as DCs, macrophages, and other monocytic system cells. CD40L as the ligand of CD40 is mainly expressed on activated T and B cells. Activation of CD40 can stimulate the activity of antigen-presenting cells, thus further activate the function of cytotoxic T cells to tumors and reverse the immunosuppression microenvironment ultimately. CD40 agonists can enhance the antitumor effects of other immunomodulators such as TLR agonists, cytokines (IFN and IL-2), adoptive immunotherapy, and chemotherapy [129]. It was found that the combination of CSF-1R blocker and CD40 agonist can significantly improve the antitumor ability and survival rate in the mouse. The Ly-6C low F4/80 $^{+}$ TAMs in the TME decreased significantly, while the remaining macrophage phenotype changed from MHC-II low to MHC-II high , which is the proinflammatory macrophage phenotype, and the expression of costimulatory molecule CD80 and CD86 increased. The combination therapy confirmed the importance of removing the inhibitory cell population and activating the surface activation signal of immune cells [130].

ADC-1013 is a highly specific humanized IgG1 antibody designed for tumor immunity, and it targets the costimulatory receptor CD40 expressed on the surface of antigen-presenting cells. ADC-1013 can activate CD40 of DC cells and further activate T cells to enhance the immune system's ability to eliminate tumors. ADC-1013 in combination with PD-1 inhibitors had a synergistic antitumor effect in bladder cancer models [131]. At present, in addition to ADC-1013, a variety of CD40 agonists have been applied in clinical trials. Selicrelumab is a humanized IgG1 agonist of CD40, which promotes T cell-driven tumor killing by activating CD40 on antigen-presenting cells [132, 133]. ABBV-428 is a bispecific protein therapeutic designed for mesothelin-dependent CD40 activation, which has been studied in phase I clinical trial of solid tumors [134]. APX005M has the potential to become the best-in-class CD40 agonist antibody, which can be used as a single agent or combined with other immuno-oncology drugs, targeted therapy drugs, chemotherapy, vaccines, and radiation therapy. APX005M is entering phase clinical II trials. The agonistic anti-CD40 antibody CP-870,893 was tested in an escalating phase I study and

has been well tolerated, resulting in antitumor activity [135]. RO7009789, another CD40 agonist, is currently being investigated in 4 combinatorial clinical trials in advanced stage solid tumors (NCT02760797, NCT02304393, NCT02665416, and NCT025843).

Targeting the TLR for TAM Reprogramming

TLRs are a class of pattern recognition receptors that improve immune system function through multiple signaling, activating NF- κ B signaling pathways and regulating the secretion of multiple cytokines such as TNF- α , ILs, and IFN- α . Imiquimod (INN) as the first TLR-targeting agonist is one of the most successful TLR-targeting drugs for various skin tumors. INN induces IFN- α , IL-6, and TNF- α secretion through the TLR7-MyD88 signaling pathway and improves adaptive immune activity by adjusting innate immune function [136]. The clinical outcome shows that the combination of TLR agonists and other immunotherapy can greatly improve the therapeutic effect on tumors. In 2018, researchers developed a novel therapy strategy – a combination of TLR9 agonists (SD-101), anti-OX40 antibodies, and radiotherapy effectively eliminates all cancer traces in mice, even untreated metastatic lesions at the distal end [137]. The TLR-9 agonist has been studied in situ immunotherapy for lymphoma and combination with PD-1 antibodies for melanoma in clinical trials. Clinical trials showed that 29 assessable patients had no treatment-related grade 4 or severe adverse events after receiving the combination treatment; for the most part, the tumors were reduced substantially. The tumors in untreated sites of 24 patients significantly reduced, 5 of which obtained partial remission and 1 patient with complete remission. CD8⁺ and CD4⁺ T cells in TMEs increased, while follicular helper T cells and regulatory T cells (Treg) decreased [138]. In the same year, another TLR9 agonist, CMP-001, was developed and obtained the exciting phase I clinical data. CMP-001 combined with PD-1 antibodies was effective in treating patients with PD-1 ineffectiveness or drug resistance by up to 22%, and tumors had completely disappeared in some of them. This treatment has truly reversed cold and hot tumors and assaulted the PD-1/L1 antibody resistance freak circle [139].

TLRs, such as TLR3 (e.g., dsRNA, poly(I:C)), TLR4 (e.g., picibanil, LPS), TLR7 (e.g., imiquimod), TLR7/8 (e.g., R848), and TLR9 (e.g., CpG-oligonucleotide), were investigated for targeting innate immune responses against cancer [140–142]. Diversified TLR agonists have been tested in vivo and are being investigated in early clinical trials to assess the safety and efficacy of cancer patients, most frequently in combination with conventional or target thera-

pies. Maeda et al. [143] used 2 immunostimulatory compounds: the TLR7 agonist Imiquimod (IMQ) and the TLR3 agonist poly(I:C) to investigate the repolarizing ability in TAMs. It is known that the 2 compounds induce repolarization of TAMs through distinct signaling pathways: IMQ-TLR7 triggers NF- κ B via the adaptor protein MyD88, resulting in the activation of an inflammatory cascade, while poly(I:C)-TLR3 signals through the TIR-domain-containing adaptor protein, leading to IFN type I production and its target genes. The cytotoxicity assay presented that poly(I:C), but not IMQ, was effective in triggering the cytotoxic activity of tumor-conditioned macrophages against cancer cells. The outcomes revealed that poly(I:C) stimulation of tumor-conditioned macrophages is more effective than IMQ in terms of macrophage reeducation toward antitumor effectors [143].

Other Strategies to Reprogram TAMs

Histone Acetylase/Histone Deacetylase

Histone acetylase and histone deacetylase (HDAC) are enzymes that regulate chromatin structure. Acetylation of core histone proteins with endogenous HAT activity leads the chromatin helicogenic transcription factor and RNA polymerase II to bind to DNA to facilitate gene transcription. Whereas deacetylation of core histones is usually associated with transcriptional inhibition, in brief, HDAC can inhibit histone acetylation. HDAC transfers acetyl groups from histone lysine residues, causing DNA entanglement and hindering the entry of basic transcription unit protein complexes into promoter binding sites, resulting in inhibition of transcriptional function. HDAC plays an important role in inhibiting gene expression and overacetylation of core histones. In the TME, cancer cells exploit class IIA HDAC to regulate macrophage proliferation and differentiation. Typically, this enzyme puts macrophages on a “bad path” that favors tumor cell growth. The application of HDAC inhibitors is an impactful strategy to stimulate the antitumor ability of macrophages. In a mouse model of breast cancer, TMP195, as an inhibitor of Class IIA HDAC, reduced the tumor size and prevented lung metastasis, enhancing the phagocytosis of macrophages [144]. TMP195 improves the corresponding efficacy and tolerance when combined with chemotherapeutic agents or anti-PD-1 antibodies. Back in 2013, researchers found that TMP195 was able to re-educate macrophages in vitro, transforming them from “rebels” who promote tumor growth to “undercover” that could attack tumor tissues [145].

PI3K γ Signaling Pathway

The PI3K γ signaling in macrophages can control the conversion of immunosuppression and immune activation. The researchers confirmed that the overexpression of macrophage-specific PI3K γ signaling pathway promotes tumor growth, and then knocking out the PI3K γ gene or using the PI3K γ inhibitor TG100-115 significantly inhibited tumor growth in mice with lung, head, and neck squamous cell carcinoma. In the process, the number of macrophages at the tumor site did not decrease, but the secretion of immunosuppressive factors had been decreased. Inhibition of the PI3K γ signaling pathway can also promote CD8⁺ T-cell migration to the tumor site and enhance the cytotoxic function of T cells [146]. The continuously increased expression of PD1 and CTLA-4 in T cells of tumor site in immunosuppressive environment induces that the long-term use of PD1 and CTLA-4 inhibitors will develop drug resistance and the number of inhibitory bone marrow cells is significantly increased. The combination of PI3K γ inhibitor IPI-549 and PD1 or CTLA-4 inhibitor significantly improved the antitumor effect [147].

Macrophage-Based Cell Therapies: Genetically Engineered Macrophages

In addition to immunotherapeutic strategies focusing on reeducating immunosuppressive TAMs or enhancing macrophage phagocytosis, another potential immunotherapeutic approach associated with macrophages is the application of genetic engineering to augment existing macrophage behaviors or endow new functionalities [19]. In brief, the primary macrophages were cultured *in vitro*, and then the modified macrophages as effector cells were transfused to the patients to reshape the positive TME. Back in 1990, the first clinical phase I study of adoptive immunotherapy in cancer patients using monocyte-derived macrophages as effector cells was reported. The clinical outcome shows that adoptive macrophage transfer was well tolerated and mild side effects were observed after *i.p.* application [148]. The failure of early trials might have been due to lack of macrophages trafficking into the tumor or to the plasticity of macrophages, resulting in a rapid loss of the antitumor phenotype.

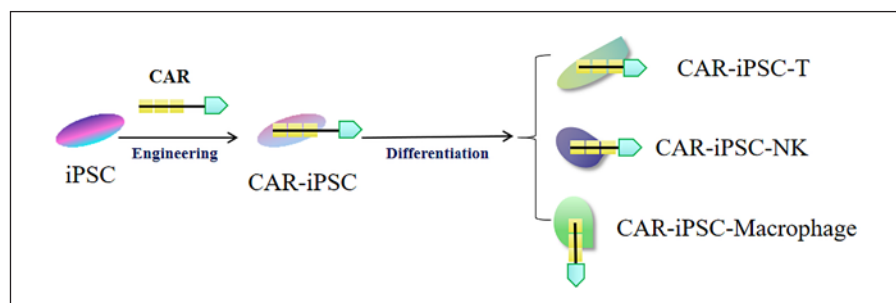
Genetic engineering methods using exogenous macrophages instead of endogenous macrophages can be used to enforce specific therapeutic behaviors. For instance, CD47 antibodies can block the “don’t eat me” signal from tumor cells while also rapidly eliminate blood

cells in the blood circulation; in addition to causing anemia, it can also lead to more serious autoimmune diseases. Researchers obtained “young and robust” macrophages from the bone marrow of healthy donors and engineered them using blocking the expression of SIRP α on the cell surface and loading them with human immunoglobulin G, the most important antibody component in the serum, which could enhance the phagocytosis of macrophages [27]. In the course of treatments, the levels of red blood cells, white blood cells, platelets, and hemoglobin in mice were kept within the normal range, and there were no serious side effects in mice. The engineered macrophages can specifically accumulate in tumor tissues and devour tumors.

Because the collagen density is relatively large in the TME, the solid tumor tissue shows a “hard” state, which will change the cell phenotype and affect the phagocytosis of macrophages [149–151]. The modified macrophages can be transformed passively to the nonphagocytic state with high expression of SIRP α after surviving around the TME in a period [27]. Therefore, how to delay the rate of assimilation of modified macrophages as much as possible is the key to remodeling adoptive macrophages. Another promising strategy of modifying macrophages is constructing macrophages with chimeric antigen receptors (CAR), which probably is more effective than CAR-T in the treatment of solid tumors. A family of chimeric antigen receptors for phagocytosis (CAR-Ps) was developed to promote macrophages to engulf cancer cells. CAR-Ps consist of an extracellular antibody fragment, which can be engineered to direct CAR-P activity toward specific antigens, giving macrophages antigen-dependent phagocytosis. By screening a panel of engulfment receptor intracellular domains, the study found that the cytosolic domains Megf10 and FcR γ robustly triggered engulfment independently of their native extracellular domain. CAR-P macrophages can reduce cancer cell numbers in an *in vitro* coculture system by over 40% [21].

CAR-T therapy has been a powerful strategy for blood cancers but has confronted straitened circumstances in targeting solid tumors. Lately, researchers adopt a macrophage-based immunotherapeutic approach that engineered primary human macrophages as a therapy for solid tumors. Unlike the therapeutic approaches for depleting, repolarizing, or derepressing the phagocytic activity of TAMs, macrophages as effectors and regulators of the innate immune system possess the ability of phagocytosis, cellular cytotoxicity, secretion of proinflammatory factors, and antigen presentation to T cells. Researchers transduced the human primary macrophages with an an-

Fig. 5. CAR-modified iPSC-derived immune cells. CAR-modified iPSC-derived cells are an excellent platform for engineering-friendly and expandable immune cells for cancer immunotherapies. CAR, chimeric antigen receptors; NK, natural killer.



ti-HER2 CAR and measured the phagocytic potential of macrophages. CAR macrophages (CAR-Ms) were capable of antigen-specific phagocytosis *in vitro* and decreased tumor burden and prolonged overall survival in the solid tumor xenograft mouse models. They also evaluated the effect of CAR-Ms on M2 macrophages *in vitro* in virtue of proinflammatory cytokines' and chemokines' expression of CAR-Ms and found that CAR-Ms can induce a phenotypic shift in M2 macrophages to M1-type and active T cells as professional antigen-presenting cells and resist the effects of immunosuppressive cytokines. It shows that genetically engineering macrophages could be the key to develop cellular therapies that effectively target solid tumors [152].

Genetically engineered primary immunocytes such as T lymphocytes, NK cells, and macrophages expressing CARs provide a promising means for gene modification to enhance antitumor properties. However, here are a few outstanding challenges for efficiently engineering primary immune cells, including low transduction efficiency, highly heterogeneous genetic outcomes upon editing the genome of the targeted cells, and limited primary cell sources. Besides, immortalized primary immune cell lines do not apply to clinical settings, thus leaving iPSC-derived immune cells as a great source for cell-based immunotherapy. iPSC-derived immune cells, due to their flexibility of expansion and genome editing at the iPSC stage, in theory, have advantages in dealing with the challenges above and have been proved to be effective in treating B cell cancer cells and ovarian cancer cells in preclinical settings such as iPSC-differentiated CAR-T cells [153] and CAR-NK cells [154]. CAR-Ms can specifically "eat" tumor cells or alter the TME, in an antigen-dependent way, providing a tool for direct phagocytosis or modulating a specific niche at the interface of tumor and immune cells. CAR-modified iPSC-derived myeloid cells may offer a novel source of off-the-shelf macrophages with antigen-specific phagocytosis, and they can also be produced on a large scale as a standardized cell product.

Thus, the iPSC-differentiated CAR-M is an excellent platform for engineering-friendly and expandable macrophage cells, and it is a valuable addition to other iPSC-differentiated immune cells for further cancer immunotherapies (shown in Fig. 5).

Conclusion

Mainstream cancer immunotherapies focus on improving the immune activation through adaptive immune systems against tumors, losing sight of the innate immune system as the powerful weapon against cancer cells. Macrophages as innate immune cells have strong phagocytosis capacity against tumor cells, and they are potent antigen-presenting cells that can activate the immune system by stimulating T cells to exert cytotoxic function. Nevertheless, most of the macrophages tend to boost the proliferation of tumor cells rather than phagocytose tumor cells. In recent years, it has emerged that macrophages can still be recreated as innate immune cells with antitumor activity instead of inhibiting tumor growth by destroying macrophages around tumor tissues. Restoring antitumor functions of macrophages is more meaningful than simply eliminating them to inhibit tumor growth. The phagocytosis and subsequent immune recognition have been increasingly recognized to be governed by multiple inhibitory and stimulatory signals that should be considered to generate optimal antitumor responses. Tumor cells tend to inhibit the functions of macrophages by expressing a variety of immunomodulatory molecules. Therefore, the discovery and application of immune checkpoints are essential to reshape macrophage functions. The CD47-SIRP α axis was the first phagocytosis checkpoint discovered in cancer, and more novel phagocytosis checkpoints have since been identified.

At present, a variety of inhibitors that block the immune checkpoint signaling pathway have been applied in

clinical treatments. Besides current macrophage-based immunotherapeutic approaches, another strategy concentrating on the combination therapy of immunocytes and immune checkpoints may greatly improve the anti-tumor effect. Independent treatment with adoptive cells is unable to prevent the occurrence of immune escape effect of tumor cells through the mechanism of immune checkpoints. As expected, the application of ICIs alone can interfere with the immunosuppression status of the TME partially and passively. For instance, compared with CAR-T therapy alone or the application of anti-PD-1 antibodies simply, the combination treatment of CAR-T and inhibitors for immune checkpoint can strengthen the antitumor effect substantially [155–160] (shown in Fig. 1).

Therefore, besides the TAMs-based immunotherapeutic approaches to repolarize TAMs to M1-like phenotype, we should attend to concentrate on macrophage-based cell therapies through genetically engineering macrophages *ex vivo* to improve the phagocytosis of macrophages and activate the tumor immune microenvironment by a large margin. Currently, research on genetic modification of macrophages is on the rise, and a handful of studies on the combination of macrophages as therapeutic cells and ICIs are being explored for oncotherapy.

The road of the combined therapy of reeducated TAM and engineered macrophages-based cell therapy *ex vivo* for oncotherapy is still long and winding. First, due to the presence of tumor heterogeneity, the current clinical application of the “do not eat me” signal is not competent to cope with most tumor cells. More immune checkpoint

signals should be developed for different types of tumors. A deeper understanding of the mechanisms that govern the potential synergy between cytotoxic agents and phagocytosis checkpoint blockers will offer key insights required for the development of effective combination regimens for cancer treatment. Second, for the engineered macrophages, primary monocytes or macrophages from patients may not sufficient for preclinical study and clinical application. Also, how to increase the efficiency of gene editing for endowing macrophages with the stronger phagocytosis or other capability is crucial for macrophages-based adoptive immunotherapy. Controlling the macrophages may bring us closer to achieving the original goals of curing cancer, but in the journey, we still have a lot of straits to confront.

Conflict of Interest Statement

J.Z. is the scientific co-founder of CellOrigin Inc. J.Z., L.T., and A.L. hold patents related to iPSC-derived CAR-M.

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Author Contributions

J.Z., L.T., and H.M. designed the manuscript; L.T. and A.L. wrote the manuscript and designed the figures; and T.T., M.Z., and L.Z. revised the manuscript.

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